

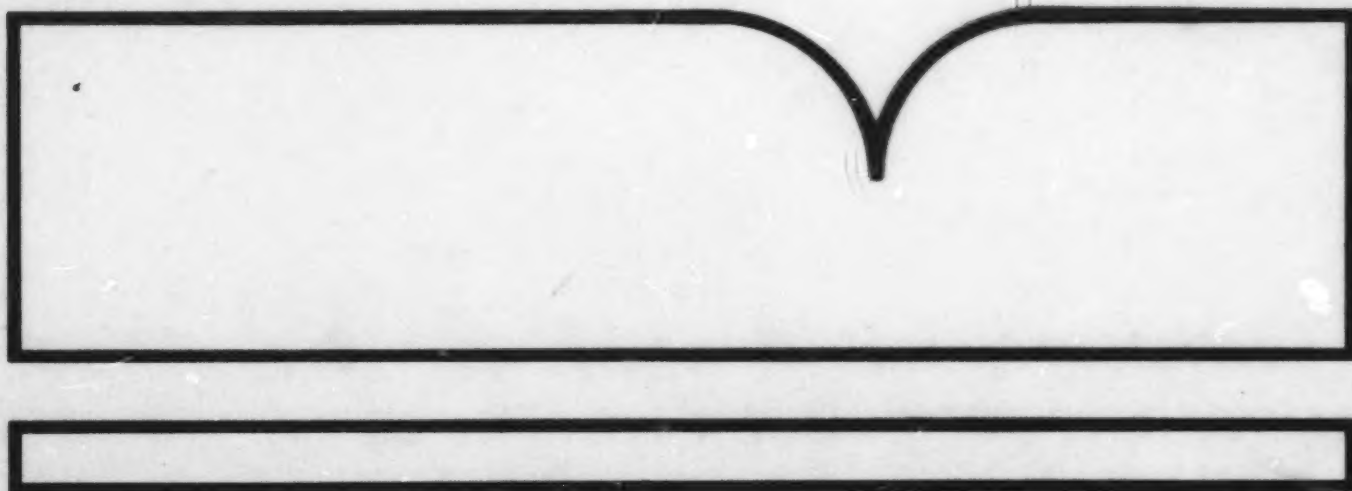
Colonization Potentials of Male and
Female E. coli K 12 Strains E coli B and
Human Fecal E. coli Strains in the
Mouse GI Tract

Rhode Island Univ.
Kingston

Prepared for

Health Effects Research Lab.
Cincinnati, OH

1980



TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA-600/J-80-222	2. Journal Article	3. RECI PR81-166597
4. TITLE AND SUBTITLE Colonization Potentials of Male and Female E. Coli K 12 Strains E. coli B and Human Fecal E. coli Strains in the Mouse GI Tract.	5. REPORT DATE	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Cohen, Pilsuck, Myhal, Rosen, Laux and Cabelli	8. PERFORMING ORGANIZATION REPORT NO.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Rhode Island Department of Microbiology Kingston, Rhode Island 02881	10. PROGRAM ELEMENT NO.	11. CONTRACT/GRANT NO. R805488-01
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory 26 W. St. Clair Street Cincinnati, Ohio 45268	13. TYPE OF REPORT AND PERIOD COVERED	14. SPONSORING AGENCY CODE
15. SUPPLEMENTARY NOTES Published in: NIH Recombinant DNA Bulletin - 1980 <i>the authors</i>		
16. ABSTRACT In order to compare the colonization potentials of individual E. coli strains, we developed a simple animal system in which both freshly isolated human strains and laboratory strains (i.e. E. coli B and E. coli K 12 strains) survive in the large intestine for long periods of time when fed to these animals alone. Once this condition was achieved the colonization potential of each E. coli strain relative to others was determined by feeding the animals two or more strains simultaneously. In the present investigation we report that mice whose normal facultative microflora is reduced by the addition of streptomycin to their drinking water can be used to compare the intrinsic large intestine colonization potentials of E. coli strains, including E. coli B male and female strains of E. coli K 12, and E. coli strains recently isolated from human feces. <i>the authors</i> <i>Escherichia</i>		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA 22161		
18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

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Colonization Potentials
of Male and Female *E. coli* K 12 Strains
E. coli B and Human Fecal *E. coli* Strains
in the Mouse GI Tract

by

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Escherichia coli K 12 strains have been fed to normal humans, mice, rats, chickens, pigs, and calves (7, 11, 15, 16). These strains have not survived in the large intestines of these animals for long periods of time. Moreover, an *E. coli* strain isolated from the feces of a human when fed back to that same human does not survive for more than a few days in his large intestine (4). Together, these data suggest that an animal's indigenous microflora has a selective advantage over strains introduced from without. In support of this view, when axenic mice were fed *E. coli* X1666 (a K 12 strain) it colonized the large intestine and persisted even when the mice were conventionalized (9). This result argues that given the proper conditions, K 12 strains have the capacity to colonize the large intestine of warm-blooded animals.

E. coli K 12 strains are used in recombinant DNA studies. If K 12 colonization potentials are high relative to normal human fecal *E. coli* strains, it is not difficult to imagine conditions under which such strains could become the predominant *E. coli* flora of young or debilitated individuals. It is therefore important to determine their intrinsic colonization potentials relative to human fecal *E. coli* strains.

In order to compare the colonization potentials of individual *E. coli* strains, we developed a simple animal system in which both freshly isolated human strains and laboratory strains (i.e. *E. coli* B and *E. coli* K 12 strains) survive in the large intestine for long periods of time when fed to these animals alone. Once this condition was achieved, the colonization potential of each *E. coli* strain relative to others was determined by feeding the animals two or more strains simultaneously. In the present investigation, we report that mice whose normal facultative microflora is reduced by the addition of streptomycin to their drinking water can be used to compare the intrinsic large intestine colonization potentials of *E. coli* strains, including *E. coli* B, male and female strains of *E. coli* K 12, and *E. coli* strains recently isolated from human feces.

Bacterial strains

E. coli strains designated human fecal strains

were isolated from the feces of University of Rhode Island students in 1977 by the method of Dufour et al. (9). *E. coli* B is from the collection of Dr. Paul S. Cohen. *E. coli* HfrH contains the F factor inserted at zero minutes of the *E. coli* K 12 chromosome and was obtained from Cold Spring Harbor Laboratories, Cold Spring Harbor, New York. *E. coli* J5-3, a K 12 F⁻ strain is unable to use lactose (lac) as a carbon source and requires proline (pro) and methionine (met) for growth. *E. coli* RS-2, a K 12 strain, contains the Rldrd19 plasmid which codes for resistance to ampicillin, chloramphenicol, tetracycline, streptomycin, and the sulfonimides (12). *E. coli* SP1000 contains the Flac⁺ plasmid. *E. coli* strains J5-3, RS-2, and SP1000 were obtained from Dr. Grace M. Thorne, Tufts Medical School, Boston, Massachusetts. Spontaneous streptomycin resistant (S^r), naladixic resistant (nal^r), or rifampin resistant (rif^r) mutants were selected by plating between 1×10^8 and 1×10^9 cells on Luria agar plates containing the appropriate antibiotic. Resistance is designated with a superscript r and sensitivity with a superscript s. Selective markers of the strains used in the experiments presented here are described in the legends to the tables.

Mouse colonization experiments

Thirty-five to 42 day-old male CD-1 mice (Charles River Mouse Farms, Inc.) were given drinking water containing streptomycin sulfate (5 grams per liter of sterilized distilled water) for four days, to clear their GI tracts of streptomycin-sensitive bacteria. By four days of streptomycin treatment, the number of facultative bacteria per gram feces dropped from approximately 1×10^8 to an undetectable number on Luria agar plates (i.e. < 10 cells per gram feces). *E. coli* is not part of the normal fecal flora of CD-1 mice (unpublished observations). The mice were then starved overnight and the next morning sets of three were fed approximately 1×10^{11} cells each of the *E. coli* strains to be tested. The bacteria were fed to each mouse in one ml of sterile 20% (w/v) sucrose. The mice ate the bacteria almost immediately and were then returned to their normal diet (Charles River Valley Rat, Mouse, Hamster Formula) including sterile

drinking water containing streptomycin. At intervals thereafter, one gram of feces was collected from each mouse, homogenized in ten ml of Luria broth, diluted, and plated on the appropriate selective media.

Prior to using a *S'* *rif*^r mutant or a *S'* *nal*^r mutant as a test strain, its ability to colonize the mouse intestine in competition with its *S'* *rif*^r or *S'* *nal*^r parent was determined. A *S'* *rif*^r mutant or a *S'* *nal*^r mutant was used as a test strain only if it colonized the mouse large intestine as well as its *S'* *rif*^r or *S'* *nal*^r parent. All strains used in this study colonize the mouse intestine at a level of about 1×10^8 cells per gram feces for at least two weeks when fed to mice in the absence of any competitor strain.

Colonization potentials of human fecal strains

The colonization potentials of eight *E. coli* strains, isolated from the feces of eight humans, were determined. Sets of three streptomycin treated mice were fed each of the strains alone or in combinations of two strains at a time and the number of cells of each per gram feces was determined at daily intervals. In all cases, the pairs were

competed in mice only if they were naturally resistant to each other's colicin. Of the eight human strains tested, all colonized equally well in competition experiments (i.e. at a level of between 1×10^8 and 5×10^8 cells per gram feces for as long as 14 days post-feeding, data not shown).

Colonization potentials of *E. coli* B and *E. coli* J5-3

E. coli B colonized the mouse intestine at a level of about 1×10^8 cells per gram feces when fed to mice alone (data not shown). However, in competition with the human fecal strain *E. coli* F-4, by the second or third day post-feeding the number of *E. coli* B cells per gram feces was of the order of 1×10^2 to 1×10^3 , depending on the mouse (Table 1). Thereafter, the number of *E. coli* B cells per gram feces increased several orders of magnitude and remained relatively constant thereafter (Table 1). These cells were confirmed as *E. coli* B by coliphage biotyping. That is, *E. coli* B is sensitive to the entire T-series of coliphages, whereas *E. coli* F-4 is sensitive to coliphages T2, T4, and T6 but resistant to T3, T5, and T7 (data not shown). In the same mice, *E. coli* F-4 colonized at a level of about 1×10^8 cells per gram feces (Table 1).

Table 1
Colonization Potential of *E. coli* B.
Number of cells per gram feces

Days Post-feeding	Mouse #1		Mouse #2	
	<i>E. coli</i> B	<i>E. coli</i> F-4	<i>E. coli</i> B	<i>E. coli</i> F-4
1	3.0×10^6	1.5×10^9	1.1×10^6	8.7×10^9
2	1.3×10^3	9.8×10^7	1.2×10^3	2.7×10^9
3	9.0×10^2	2.3×10^8	$<1.0 \times 10^2$	6.5×10^8
7	8.3×10^4	3.5×10^7	1.1×10^7	6.5×10^7
9	3.1×10^6	1.6×10^8	4.4×10^7	1.8×10^8
14	1.0×10^8	1.1×10^8	3.2×10^7	1.2×10^8

**E. coli* F-4 *S'* and *E. coli* B *S'* *rif*^r were fed to mice. Fecal samples were spread on Luria-agar containing either 100 μ g/ml streptomycin sulfate or containing both 100 μ g/ml streptomycin sulfate and 50 μ g/ml rifampin. For brevity, data from only two of the three mice tested are shown.

To determine whether the mouse intestine selected genetic variants of *E. coli* B that were better colonizers than the parent strain, mice were fed both *E. coli* F-4 and an *E. coli* B strain that had been reisolated from mouse feces. The same kinetics of *E. coli* B colonization were observed as in the initial experiment (data not shown). Therefore *E. coli* B is genetically the same before and after passage through the mouse intestine and presumably adapts to the intestine in competition with *E. coli* F-4. Essentially the same results as reported above were observed when *E. coli* B was

competed with *E. coli* F-25, another of the recently isolated human fecal strains (data not shown).

E. coli J5-3 is a K12 F⁻ strain. It also colonized at a level of about 1×10^8 cells per gram feces when fed alone to mice (data not shown). In competition with *E. coli* F-4, *E. coli* J5-3 colonized well throughout the duration of the experiment leveling off at approximately 1×10^7 cells per gram feces (Table 2). Apparently *E. coli* J5-3 does not require the adaption period to the intestine that is necessary for *E. coli* B colonization.

Table 2
Colonization Potential of *E. coli* J5-3^a
Number of cells per gram feces

Days Post-feeding	Mouse #1		Mouse #2	
	<i>E. coli</i> J5-3	<i>E. coli</i> F-4	<i>E. coli</i> J5-3	<i>E. coli</i> F-4
1	3.2×10^7	2.8×10^8	1.5×10^7	3.0×10^8
2	7.0×10^6	1.0×10^9	1.5×10^7	2.3×10^8
3	1.5×10^6	8.0×10^7	2.2×10^7	2.8×10^8
4	1.8×10^7	7.5×10^7	2.3×10^7	3.5×10^8
7	9.5×10^7	2.3×10^8	4.8×10^6	1.2×10^8
10	1.9×10^7	1.1×10^8	1.8×10^7	8.0×10^8
14	2.5×10^8	1.0×10^8	5.0×10^8	8.5×10^7

^a*E. coli* F-4 S^r rif^r nal^r and *E. coli* J5-3 S^r rif^r nal^r were fed to mice. Fecal samples were spread on Luria-agar plates containing either 100 µg/ml streptomycin sulfate and 50 µg/ml streptomycin sulfate or 100 µg/ml streptomycin sulfate and 100 µg/ml naladixic acid.

Colonization potential of *E. coli* J5-3 containing the *Flac*⁺ plasmid

It has been reported that piliated *E. coli* strains survive less well in the human intestine than their non-piliated counterparts (17). To test whether the

same is true in mice, the following experiment was performed. *E. coli* J5-3 lac⁻ cells, containing the *Flac*⁺ plasmid, were competed in mice with an equal number of *E. coli* F-4 cells. The *Flac*⁺ containing J5-3 strain was found to compete very well with F-4 (Table 3).

Table 3
Colonization of *E. coli* J5-3 lac⁻ containing the *F lac*⁺ plasmid^a
Number of cells per gram feces

Days Post-feeding	Mouse #1			Mouse #2		
	<i>E. coli</i> J5-3	%I2 resistant J5-3b	<i>E. coli</i> F-4	<i>E. coli</i> J5-3	%I2 resistant J1-5b	<i>E. coli</i> F-4
1	6.5×10^8	0	1.5×10^{10}	2.2×10^8	0	5.8×10^8
2	8.0×10^7	0	2.8×10^9	3.0×10^8	0	2.1×10^9
3	2.5×10^7	0	3.2×10^8	1.5×10^7	0	4.2×10^8
4	8.3×10^6	0	1.1×10^8	9.0×10^6	0	2.2×10^8
5	1.3×10^7	0	6.0×10^7	2.8×10^7	10	1.8×10^8
6	5.0×10^6	30	3.5×10^7	2.4×10^7	40	3.8×10^8
7	6.5×10^6	60	2.2×10^7	1.3×10^7	60	2.3×10^8
10	2.2×10^7	80	5.5×10^7	5.5×10^6	80	7.5×10^8
11	5.5×10^6	90	7.5×10^7	2.3×10^7	100	2.3×10^8

^a*E. coli* F-4 S^r rif^r nal^r and *F lac*⁺ containing *E. coli* J5-3 lac⁻ S^r rif^r nal^r were fed to mice. Fecal samples were plated as in the legend to Table 2.

^bTen S^r rif^r nal^r colonies were tested for I2 sensitivity each day.

However, by 5 days post-feeding, bacteriophage I2 resistant J5-3 colonies were observed. These colonies were found to be lac⁻ and able to accept the *Flac*⁺ plasmid at the same frequency as J5-3, showing that they were indeed F⁺ (Table 4). By day 11, greater than 90 percent of the J5-3 cells present in mouse feces were F⁺ (Table 3). In control experiments it was shown that the concentration of streptomycin fed to the mice did not cause

segregation of the *Flac*⁺ plasmid from J5-3 cells. That is, in seven successive 24 hr. subcultures (beginning at 10⁸ cells per ml at each subculture) in Luria broth containing 5 mg per ml streptomycin sulfate, the frequency of F⁺ cells in the *Flac*⁺ plasmid containing population remained at approximately 2 x 10⁻¹. Together these experiments suggested that the mouse large intestine selected F⁺ cells for colonization over the same cells containing the *Flac*⁺ plasmid.

Table 4
Ability of *E. coli* J5-3 lac⁻ strains isolated from mouse feces
to accept the Flac⁺ plasmid^a

Flac ⁺ Donor	Recipient	Flac ⁺ transcripts per donor cell
<i>E. coli</i> SP1000	<i>E. coli</i> J5-3 lac ⁻	3.1×10^2
<i>E. coli</i> SP1000	<i>E. coli</i> J5-3 lac ⁻ (isolated from Mouse #1) ^b	2.5×10^2
<i>E. coli</i> SP1000	<i>E. coli</i> J5-3 lac ⁻ (isolated from Mouse #2) ^b	2.0×10^2

^a*E. coli* SP1000 S^r nal^r was mated with each of the S^r nal^r resistant recipients. Samples were spread on Venus-agar containing 100 µg/ml streptomycin sulfate and 100 µg/ml naladixic acid. F lac⁺ transcripts grow up as yellow colonies. Lac⁻ colonies are white.

^bIsolated from Mouse #1 and Mouse #2 of Table 3 on day 10 post-feeding.

To test the relative colonization potentials of J5-3 lac⁻ and J5-3 lac⁻ containing the Flac⁺ plasmid more directly, the following experiment was performed. Mice were fed *E. coli* F-4, and equal amounts of J5-3 lac⁻ and J5-3 lac⁻ containing the Flac⁺ plasmid. Under these conditions, the ratio of J5-3 lac⁻ cells to J5-3 lac⁻ cells containing the

Flac⁺ plasmid in mouse feces increased logarithmically throughout the course of the experiment (Table 5). Clearly, the non-piliated *E. coli* J5-3 lac⁻ was selected for immediately over its pilated counterpart by the mouse intestine and is therefore the better colonizer of the two strains.

Table 5
Relative Colonization Potentials of *E. coli* J5-3 lac⁻
containing the F lac⁺ plasmid and *E. coli* J5-3 lac⁻

Days post-feeding	Ratio of J5-3 lac ⁻ to J5-3 lac ⁻ containing F lac ⁺	
	Mouse #1	Mouse #2
1	3.4	2.8
2 ^a	11.0 (11.0)	---
3	55.6 (58.0)	13.6 (25.0)
4	184 (180)	57.1 (80.0)
5	670 (750)	393 (270)
6	3600 (3000)	595 (800)

^a*E. coli* F-4 S^r rif^r nal^r (1×10^{10} cells), F lac⁺ containing *E. coli* J5-3 lac⁻ S^r rif^r nal^r (5×10^9 cells) and *E. coli* J5-3 lac⁻ S^r rif^r nal^r (5×10^9 cells) were fed to mice. Samples were spread on Luria-agar containing 100 µg/ml streptomycin sulfate and 50 µg/ml rifampin and on Venus-agar containing 100 µg/ml streptomycin and 100 µg/ml naladixic acid. Values in parentheses are taken from the best fit line generated from a plot of the log 10 of the ratio of J5-3 lac⁻ to J5-3 containing the F lac⁺ plasmid vs days post-feeding.

Colonization potential of *E. coli* HlrH

E. coli HlrH is a pilated K 12 strain which has the F factor inserted at minute zero of the chromosome and is sensitive to bacteriophage I2 and the entire T-series of coliphages, (5). In view of the results with *E. coli* J5-3 colonization, it seemed possible that the pilated HlrH strain might be

selected against in the mouse intestine and be replaced by non-piliated derivatives of the strain. This possibility was realized. When mice were fed *E. coli* F 4 and *E. coli* HlrH, by six days post-feeding 20 to 30 percent of the HlrH cells isolated from feces were bacteriophage I2 resistant and by day ten 100% were I2 resistant (Table 6).

Table 6
Colonization Potential of *E. coli* HfrH
Number of cells per gram feces^a

Days post-feeding	Mouse #1			Mouse #2		
	<i>E. coli</i> HfrH	%I2 resistant HfrHs	<i>E. coli</i> F-4	<i>E. coli</i> HfrH	%I2 resistant HfrHs	<i>E. coli</i> F-4
1	2.2 x 10 ⁸	0	1.2 x 10 ¹⁰	1.6 x 10 ⁸	0	1.8 x 10 ⁸
2	7.5 x 10 ⁷	0	6.0 x 10 ⁹	1.8 x 10 ⁸	0	4.2 x 10 ⁷
3	5.0 x 10 ⁷	0	6.5 x 10 ⁹	4.0 x 10 ⁸	0	1.0 x 10 ⁸
6	4.5 x 10 ⁷	30	2.5 x 10 ⁹	4.8 x 10 ⁸	20	1.2 x 10 ⁸
7	5.0 x 10 ⁷	50	7.5 x 10 ⁹	2.5 x 10 ⁸	20	8.5 x 10 ⁷
8	5.5 x 10 ⁷	100	8.0 x 10 ⁹	3.0 x 10 ⁸	90	5.3 x 10 ⁷
9	6.5 x 10 ⁷	100	1.2 x 10 ⁹	1.3 x 10 ⁷	70	2.1 x 10 ⁸
10	1.3 x 10 ⁸	100	2.0 x 10 ⁹	8.0 x 10 ⁷	100	2.1 x 10 ⁸
13	5.5 x 10 ⁷	100	1.3 x 10 ⁹	2.0 x 10 ⁸	100	9.5 x 10 ⁷

^a*E. coli* F-4 S⁻ rif^r and *E. coli* HfrH S⁻ rif^r were fed to mice. Fecal samples were spread on Luria-agar containing 100 µg/ml streptomycin sulfate. Daily, each of 1000 colonies were picked from these plates to a Luria agar plate containing streptomycin sulfate (100 µg/ml) and to an identical spot on a Luria-agar plate containing streptomycin sulfate (100 µg/ml) and rifampin (50 µg/ml) and after incubation at 37° the fraction of rif^r (HfrH) and rif^r (F-4) colonies were scored.

^bTen S⁻ rif^r colonies were tested for I2 sensitivity each day. I2 resistant colonies were confirmed as HfrH derivatives by T-series coliphage biotyping. In mice fed *E. coli* F-4 S⁻ rif^r alone, no S⁻ rif^r colonies were found in 1000 colonies tested between days 7 and 13 post-feeding.

These I2 resistant strains were found to be F phenocopies and not true F strains. Like true F strains, they were unable to act as chromosome donors (Table 7). However, unlike true F strains, the F phenocopies were unable to accept and

then donate the Flac⁺ plasmid (Table 8) although they were able to accept and express the F pili of the F factor compatible Rldrdi9 plasmid at a frequency approaching 1.0 per donor cell (data not shown).

Table 7
THE INABILITY OF I2 RESISTANT HfrH STRAINS (F PHENOCOPIES), ISOLATED FROM MOUSE FECES, TO DONATE THEIR CHROMOSOMES^a

pro ⁻ nal ^s donor	pro ⁻ nal ^s recipient	Number of pro ⁻ nal ^s recombinants donor cell ^b
<i>E. coli</i> HfrH	<i>E. coli</i> J5-3	4.2 x 10 ⁻¹
<i>E. coli</i> F phenocopy (isolated from mouse #1)	<i>E. coli</i> J5-3	<3.8 x 10 ⁻⁸
<i>E. coli</i> F phenocopy (isolated from mouse #2)	<i>E. coli</i> J5-3	<4.6 x 10 ⁻⁸

^aThe F phenocopies are the same as those in Table 6. One ml of the mating mixture was washed twice and grown overnight in 20 ml of Davis medium containing dextrose (10 g/l), proline (50 µg/ml), and methionine (50 µg/ml). pro⁻ nal^s recombinants and total cells were then quantified on Davis minimal-agar containing glucose, methionine and naladixic acid (100 µg/ml) and Davis minimal-agar containing glucose, methionine, and proline respectively.

^bThe number of pro⁻ nal^s recombinants per donor cell was calculated on the ratio of donors to recipients in the original mating mixture.

Table 8
The inability of F⁻ phenocopies to accept the F⁺ lac⁺ plasmid

Passage of F ⁺ lac ⁺	F ⁺ lac ⁺ transipients per 10 ⁸ cells ^a
From SP1000 to J5-3 lac ⁺ to J5-3 lac ⁺	490
From SP1000 to F ⁻ phenocopy #1 to J5-3 lac ⁺	<0.23
From SP1000 to F ⁻ phenocopy #2 to J5-3 lac ⁺	<1.4

^a*E. coli* SP1000 S⁺ nal^r (1.0 x 10⁸ cells/ml) was mated with *E. coli* J5-3 lac⁺ S⁺ nal^r (3.5 x 10⁸ cells/ml) and the same S⁺ nal^r F⁻ phenocopies isolated for the experiment reported in Table 6. The mating mixtures were regrown from 1 x 10⁶ cells/ml at 37° in Luria broth containing 100 µg/ml streptomycin sulfate to eliminate SP1000 as a donor, and then grown from 1 x 10⁶ cells/ml to about 9.0 x 10⁸ cells/ml in Penassay broth. These cultures were then mated in equal volume with *E. coli* J5-3 lac⁺ S⁺ nal^r (8.0 x 10⁸ cells/ml). Platings were made on Venus-agar containing 100 µg/ml streptomycin sulfate and 100 µg/ml naladixic acid (see legend to Table 4).

The simplest explanation for these results is that the F⁻ phenocopies have lost some F factor genes, but have retained F factor incompatibility genes (8). That the F⁻ phenocopies may have lost much of the F factor was further suggested by the fact that they were found not to have heat labile sur-

face antigen(s) (i.e. presumably the surface exclusion proteins and F pili (1,6) present on their HfrH parents (Table 9)). In any case, it is clear that the mouse intestine selected functionally non-piliated derivatives of the *E. coli* HfrH strain for long term colonization (Table 6).

Table 9
Effects of adsorption with an F⁻ phenocopy on the reactivity of antiserum prepared against *E. coli* HfrH^a

Agglutination titer of antiserum tested against:

Antiserum	HfrH		F ⁻ phenocopy	
	Unheated ^b	heated ^b	Unheated ^b	heated ^b
unadsorbed anti-HfrH	10,240	640	5,120	640
F ⁻ phenocopy adsorbed anti-HfrH ^c	320	— ^d	— ^d	— ^d

^aThe F⁻ phenocopy is the same strain as in Table 6, isolated from mouse #1.

^bHeat stable antigen preparations were obtained by heating suspensions of the bacteria for 2.5 hr. at 100°.

^cAntiserum was adsorbed repeatedly with both heated and unheated F⁻ phenocopy cells.

^d— not detectable.

Discussion

E. coli B, a plasmidless laboratory strain, is initially an extremely poor colonizer of the mouse intestine relative to the human fecal strain *E. coli* F-4 (Table 1). However, within 3 to 4 days post-feeding, *E. coli* B adapts to the mouse intestine, multiplies, and finally colonizes at a level only slightly lower than *E. coli* F-4 (Table 1). In contrast, *E. coli* J5-3, AK 12 F strain, does not require a period of adaptation to the mouse intestine prior to colonizing at a level only slightly below that of *E. coli* F-4 (Table 2). One explanation for this difference is that *E. coli* B colonization potential is inducible whereas *E. coli* J5-3 colonization potential is constitutive. Alternatively, it is possible that *E. coli* J5-3 has a greater affinity for colonization sites in the mouse intestine than *E. coli* B, but that both strains, once attached to those sites, multiply equally well.

We have shown that pilated F factor-containing K 12 strains do not colonize the mouse intestine as well as their plasmidless counterparts (Tables 3, 5 and 6). Whether this is due specifically to pili or to other F factor directed surface antigens (Table 9) is still open to question. In any case, the data presented here are in agreement with those of Anderson who showed that R factor-containing *E. coli* strains survive less well in the human intestine than their R factorless counterparts (3). Moreover, the data presented here are in agreement with those reported by Smith and Huggins (17) who showed that a derepressed F factor-containing *E. coli* strain (i.e. pilated) survived less well in two humans than the same strain carrying the repressed F factor (i.e. non-pilated). It therefore appears that in this respect the mouse intestine is a good model of the human intestine. It should also be noted that other workers have also found the mouse intestine to be a selective environment for specific traits in bacteria. Miller et al. reported that smooth forms of *Vibrio cholerae* are selected for in the intestines of gnotobiotic mice inoculated with rough forms (13).

The results of our experiments also suggest that *E. coli* strains recently isolated from human feces and *E. coli* K 12 strains do not compete with each

other directly in the mouse intestine. That is, *E. coli* F-4 and the Flac⁺ plasmid containing *E. coli* J5-3 lac⁻ strain coexist equally well in the mouse intestine for six days (Table 3). After that time, plasmidless J5-3 lac⁻ reaches a population density whereby it outcompetes Flac⁺ containing J5-3 lac⁻ cells for colonization of the mouse intestine (Table 3). However, in the same animals, J5-3 lac⁻ does not outcompete *E. coli* F-4 (Table 3). Therefore, human fecal strains and K 12 strains must have different requirements for effective colonization. Whether these different requirements reflect different attachment sites in the mouse intestine is still open to question. Alternatively, Flac⁺ containing J5-3 lac⁻ and J5-3 lac⁻ may compete for a limiting nutrient in the intestine which *E. coli* F-4 does not require. The same argument would also apply to competition between *E. coli* F-4 and *E. coli* HfrH (i.e. significant numbers of F⁻ phenocopies emerge in the feces of mice fed these strains six days post-feeding without effecting the numbers of *E. coli* F-4) (Table 6). Moreover, it is possible that *E. coli* B and *E. coli* F-4 occupy different sites in the mouse intestine since after an initial drop *E. coli* B adapts to the mouse intestine, but not at the expense of *E. coli* F-4 (Table 1).

It should be emphasized that under the proper conditions (e.g. antibiotic pressure) K 12 strains are good colonizers relative to *E. coli* strains recently isolated from human feces (Tables 2 and 6). Since wild type K 12 strains are used as vehicles for replication of recombinant DNA, it is especially important to determine those factors necessary for K 12 colonization. Once these factors are known, they can be engineered out of the K 12 strains used in recombinant DNA research.

Finally, while the mouse model presented here does not mimic either the "normal" human intestine or the "normal" mouse intestine, it can be used to determine relative colonization potentials of individual *E. coli* strains. Moreover, this simple system should prove useful in determining the molecular basis of observed differences among these strains (Table 9).

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ACKNOWLEDGMENTS

This investigation was supported by grant R805488010 from the Environmental Protection Agency to PSC. We are greatly indebted to Ms. Dayl Mongeon for excellent technical assistance